Effects of Psychosocial Stress on DHEA and DHEA-S levels

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Effects of Psychosocial Stress on DHEA and DHEA-S levels
Acute and Long-term effects

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ABSTRACT

Background: Long-term psychosocial stress can cause and contribute to a wide range of psychological and somatic conditions, and accelerate aging. One of the consequences of long-term psychosocial stress may be a reduction in the levels of dehydroepiandrosterone and its sulphated metabolite dehydroepiandrosterone sulfate (DHEA-S). The aim of this thesis was to investigate the effects of acute and long-term psychosocial stress on serum levels of DHEA and DHEA-S in otherwise healthy men and women. Method: In Paper I, 39 healthy individuals went through a stress test (Trier social stress test). Blood samples were collected before the stress test, immediately after the stress test and after 30 minutes of recovery. Mixed between-within ANOVAs were used to investigate the responses of DHEA and DHEA-S. Thirty-six of the 39 participants in Paper I answered a questionnaire regarding long-term stress (perceived stress at work) and were included in Paper II. DHEA and DHEA-S response during acute stress were compared between groups of individuals who reporting different levels of long-term stress (Low, Medium, High) using ANCOVA. The Low stress group, which did not experience any stress at work, was used as reference group. In Paper III, morning DHEA-S and DHEA levels were measured in serum in 41 stressed and 40 non-stressed individuals. The groups were defined based on their scores on the questionnaire measuring long-term stress (perceived stress at work). DHEA and DHEA-S levels were compared between the groups using ANCOVA. Results: While acute psychosocial stress increases the levels of DHEA and DHEA-S temporarily (Paper I), long-term psychosocial stress is associated with reduced capacity to produce DHEA-S during acute stress (Paper II) and lower basal DHEA-S levels (Paper III). Conclusions: Considering the beneficial effects that DHEA and DHEA-S have and the fact that low DHEA and DHEA-S levels are associated with adverse health, the findings of this thesis suggest that one of the links between long-term stress and adverse health could be that long-term stress reduces the capacity to produce DHEA-S.

Keywords: Psychosocial stress; Acute and Long-term stress; Work stress; DHEA; DHEA-S

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SVENSK SAMMANFATTNING

Bakgrund: Långvarig stress kan orsaka och bidra till en mängd olika sjukdomar och ohälsotillstånd, samt påskynda åldrandet. En av konsekvenserna av långvarig stress kan vara sänkta nivåer av dehydroepiandrosteron (DHEA) och dehydroepiandrosteron-sulfat (DHEA-S). Syftet med denna avhandling var att undersöka effekterna av akut och långvarig stress på nivåerna av DHEA och DHEA-S i blodet. Metod: I Delarbete I genomgick 39 friska män och kvinnor i åldern 30-50 år ett stresstest (Trier Social Stress test). Blodprov togs före stresstestet, direkt efter stresstestet och efter 30 minuters återhämtning. Mixed between-within ANOVA användes för att undersöka förändringar i nivåer av DHEA och DHEA-S. 36 av de 39 studieindividerna i Delarbete I hade besvarat en enkät angående långvarig stress (upplevd stress på arbetet) och inkluderades i Delarbete II. Med hjälp av ANCOVA jämfördes DHEA- och DHEA-S-respons under akut stress mellan grupper av individer som rapporterat olika nivåer av långvarig stress (Låg, Medium och Hög stress). Låg-stress-gruppen, vilken inte upplevde någon stress på arbetet, användes som referensgrupp. Av 172 friska män och kvinnor i åldern 25-50 år som svarat på en enkät angående långvarig stress (upplevd stress på arbetet) inkluderades de som rapporterade att de inte upplevde stress (n = 40) och de som upplevde stress (n = 41) (högsta och lägsta kvartilen) i Delarbete III. Nivåer av DHEA-S och DHEA mättes i serumprov taget på morgonen och nivåerna jämfördes mellan den stressade och den icke-stressade gruppen med ANCOVA. Resultat: DHEA- och DHEA-S-nivåerna ökade under akut psykosocial stress (Delarbete I), medan de med långvarig stress uppvisade lägre DHEA-S produktion under akut stress (Delarbete II) och lägre basala nivåer av DHEA-S (Delarbete III). Slutsatser: Med tanke på de positiva effekter och skyddande funktioner som DHEA och DHEA-S har, och det faktum att låga nivåer av DHEA och DHEA-S förknippas med dålig hälsa, tyder fynden i denna avhandling på att minskad förmåga att producera DHEA-S skulle kunna utgöra en länk mellan psykosocial stress och ohälsa.

Nyckelord: Psykosocial stress; Akut stress; Långvarig stress; Arbetsrelaterad stress; DHEA; DHEA-S

LIST OF PAPERS

This thesis is based on the following papers, referenced in the text by their Roman numerals I-III:

I. **Lennartsson AK**, Kushnir MM, Bergquist J, Jonsdottir IH. DHEA and DHEA-S response to acute psychosocial stress in healthy men and women. *Biological Psychology* 2012 May;90(2):143-9. Epub 2012 Mar 13.

II. **Lennartsson AK**, Theorell T, Kushnir MM, Bergquist J, Jonsdottir IH. Perceived stress at work is associated with attenuated DHEA-S response during acute psychosocial stress. *Psychoneuroendocrinology* 2013 Feb 18 [Epub ahead of print].

III. **Lennartsson AK**, Theorell T, Rockwood A, Kushnir MM, Jonsdottir IH. Perceived stress at work is associated with lower levels of DHEA-S. *Submitted for publication*

Related papers not included in the thesis:

Lennartsson AK, Jonsdottir IH. Prolactin in response to acute psychosocial stress in healthy men and women. *Psychoneuroendocrinology*. 2011 Nov;36(10):1530-9. Epub 2011 May 28.

Lennartsson AK, Kushnir MM, Bergquist J, Billig H, Jonsdottir IH. Sex steroid levels temporarily increase in response to acute psychosocial stress in healthy men and women. *International Journal of Psychophysiology*. 2012 Jun;84(3):246-53. Epub 2012 Mar 9.

ABBREVIATIONS

3β-HSD	3-β-hydroxysteroid dehydrogenase
ACTH	Adrenocorticotropic hormone
BMI	Body Mass Index
CRH	Corticotropic releasing hormone
CYB5	Cytochrome b5
CYP11A1	Cytochrome P450SCC (cholesterol side-chain cleavage enzyme)
CYP11B1	Cytochrome P450 11B1 (11β-hydroxylase)
CYP17A1	Cytochrome P450 17A1 (17α-hydroxylase/17,20 lyase)
CYP21A1	Cytochrome P450 CYP21A1 (21-hydroloxylase)
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulphate
HPA axis	Hypothalamic–pituitary–adrenal axis
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
NK cells	Natural killer cells
SE Questionnaire	Stress-Energy Questionnaire
SNS	Sympathetic nervous system
StAR	Steroidogenic acute regulatory protein
STS	Steriod sulfatase enzyme
SULT2A1	DHEA sulfotransferase
TSST	Trier Social Stress Test
ZR	Zona reticularis

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INTRODUCTION

Psychosocial stress is a major public health problem [1-3]. Long-term psychosocial stress can cause and contribute to different diseases and symptoms, and accelerate aging [4-8]. In studies on physiological effects of psychosocial stress the main focus has been on catabolic processes, in particular the activity of the hypothalamic-pituitary-adrenal (HPA) axis through assessment of cortisol levels. Cortisol stimulates mobilization of the energy needed to overcome the stressor. As cortisol is a hormone with mainly catabolic effects, high levels can also result in damaging bodily processes. Cortisol is synthesized by the adrenal cortex in response to secretion of adrenocorticotropic hormone (ACTH). Dehydroepiandrosterone (DHEA) and its sulphated metabolite dehydroepiandrosterone sulfate (DHEA-S) are hormones also secreted by the adrenal cortex in response to ACTH [9]. While cortisol´s main role is catabolic, DHEA and DHEA-S have anabolic effects. Thus they have a protective and regenerative role. Contrary to cortisol, DHEA and DHEA-S have received little attention within the stress research area and published studies on the relationship between stress and DHEA and DHEA-S are few. One of the consequences of long-term psychosocial stress may be a reduction in DHEA and DHEA-S levels. If so, low DHEA-S and DHEA levels could constitute one link between psychosocial stress, ill health and accelerated ageing. The aim of this thesis was to study the effects of psychosocial stress on DHEA and DHEA-S. The first part of the introduction contains a brief explanation of stress, in particular psychosocial stress, and how stress can cause adverse health. Thereafter, the hormones DHEA and DHEA-S are described, in the context of their biosynthesis, regulation, and functions. Finally, the introduction will lead into the idea and importance of studying DHEA and DHEA-S in relation to psychosocial stress.

Psychosocial stress

The term “stress” is used to describe the physiological reaction that occurs in response to demands (stressors). Different brain areas determine what is demanding and respond by inducing bodily processes that mobilize the energy needed to overcome the stressor. Psychosocial stress can be defined as the result of a cognitive appraisal of threat of psychological and social kind. In this context, the word stress implies, besides the physiological stress reaction, also the psychological (and somatic) feeling that occurs during the physiological stress reaction (e.g. stressed, tense, under pressure, loss of control). We experience psychosocial stress when we perceive threat in our

lives and we determine that these threats may require resources we do not have. Psychosocial stress could be induced by critical life events, such as job loss, divorce or loss of a loved one, and by daily hassles and life difficulties [10]. Examples of psychosocial stressors of more chronic type i.e. conditions that last for longer periods or even throughout life are financial problems, a sick family member or relative, unemployment and unresolved conflicts [10]. The work place is a commonly reported source of chronic psychosocial stress. Stressors that arise within the psychosocial work environment are for example, work overload, conflicts at work, reorganization at work, limited opportunities to influence the work situation and unfair treatment and lack of reward from the employer [11-14]. We can turn on the stress response not just by perceiving a stressor when the situation actually occurs but also by just thinking about potential stressors (things which we think may happen in the future for example). How we perceive and cope with the stressors varies greatly among individuals depending on many factors such as childhood experiences [15], personality [16] and genetics [17].

What happens in the body during acute stress?

When the brain perceives (or thinks about) a stressor, the body reacts with a stress reaction in order to cope with the stressor and overcome it. The brain stem and the hypothalamus constitute central parts of this reaction since they receive information from higher brain areas and since they activate the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis [18-20]. The sympathetic nervous system then acts by releasing the hormones/neurotransmitters adrenaline and noradrenaline from the nerve endings (including the nerve endings in the adrenal medulla) [19,20]. The other major part of the stress reaction is increased HPA axis activity and production of glucocorticoids, in particular cortisol [19,21]. In a response to an acute stressor the hypothalamus secretes corticotropic releasing hormone (CRH) [22]. CRH is transported in small vessels towards the pituitary gland, which responds to the CRH by secreting adrenocorticotropic hormone (ACTH). When it reaches the adrenal cortex through the blood stream, the production of cortisol is stimulated. Cortisol, adrenaline and noradrenaline are responsible for most of the bodily reactions that then occur. Increased levels of these hormones result in mobilization of different energy resources [23]. Glucose, proteins and free fatty acids are released from their deposits (fat cells, liver and muscles) into the blood stream. Respiration rate, heart rate and blood pressure increase, enabling the nutrition and oxygen to rapidly reach parts of the body that require more energy. Increased blood flow is directed to the brain to enhance the cognitive ability to cope with the stressor, and to the muscles in legs and arms to enhance the ability to fight or escape.

When the sympathetic nervous system is activated, the activity of the parasympathetic nervous system is inhibited, since these systems are partly competing with one another [24]. Thus, the bodily functions and organs that are less important in overcoming the stressor are given lower priority (e.g. digestion, uptake and storage of nutrition, circulation to skin and extremities, reproduction, and restorative and regenerative activities) [24]. When we perceive that the threat is over, the brain gives the signals to reduce the activity in SNS and HPA axis and the production of the stress hormones reduce to normal levels. Chronic activation of acute stress reactions without sufficient recovery can result in adverse health effects. Long-term psychosocial stress may cause or contribute to (or negatively influence the course of) a variety of psychological and somatic conditions and disease states such as stress-related exhaustion disorder [25], depression [26], anxiety [27], impaired sleep [4] cardiovascular disease [28,29], metabolic syndrome [8], type 2 diabetes [30], obesity [31,32], muscle pain [33,34], autoimmune diseases [35] and inflammatory bowel syndrome [36].

How can long-term stress cause adverse health?

The adverse health effects associated with long-term exposure to stress are partly due to the effects of long-term exposure to elevated levels of glucocorticoids and sustained, enhanced activity of the sympathetic nervous system and its release of adrenaline and noradrenaline. Elevated levels of stress hormones render the ability to cope with stressors but also induce bodily processes resulting in negative consequences for the organism. I will shortly mention some of these. Psychosocial stress increases the production of free radicals [37] and lowers the body's own antioxidative defence [38,39]. Long-term stress is associated with oxidative damage [39-42] and accelerated cell aging [6], which in turn are associated with increased risk for age-related diseases. Long-term stress inhibits the immune system [43] i.e. it reduces the number and activity of natural killer (NK) cells [44,45]. Increased levels of pro-inflammatory cytokines are also seen during long-term stress [43,46] which in turn negatively influences a range of health conditions. Long-term stress can cause damage and inflammation in endothelial cells [47,48] and this will in turn lead to formation of atherosclerotic plaques [47]. Through excess levels of glucocorticoids, chronic stress can cause atrophy of the hippocampus, which may lead to impaired memory function and disrupted negative feedback control of glucocorticoid production [49,50]. An excess of glucocorticoids can also cause atrophy of the thymus gland [51], which is one of the pathways between long-term stress and suppressed immune system. In general, it could be said that long-term stress lowers the body's regeneration activity, thus it reduces the ability to regenerate cells and

tissues [52,53]. For example, it has been shown that wound healing and recovery from injuries and surgery are delayed in stressed individuals [54-59]. Recovery, in particular recovery during sleep, is important for regeneration and restoration [53,60,61] and is a protective factor against developing adverse health effects due to long-term psychosocial stress. However, psychosocial stress often causes sleep disturbances [4,62] resulting in lack of recovery and energy restoration [61]. Thus, impaired sleep is likely an important link between long-term stress and increased vulnerability to developing stress-related adverse health. Taken together, enhanced activity of the sympathetic nervous system and elevated levels of stress hormones, in particular cortisol, induce processes that increase the risk of adverse health and accelerated ageing, if the stress is prolonged or extensive. Long-term stress can also indirectly lead to the development of adverse health through lifestyle changes, since stressed individuals are more likely to have unfavourable health behaviours (choosing unhealthy food and being sedentary for example) [63].

DHEA and DHEA-S

Biosynthesis of DHEA and DHEA-S (and cortisol)

Site of synthesis

DHEA and DHEA-S are, as well as cortisol produced in the cortex of the adrenal glands. The adrenal glands are located at the top of each kidney. The adult adrenal glands have a combined weight of 8-10 grams. The inner medulla stands for 10 % of its weight and the cortex 90 % [64]. The adrenal cortex consists of three layers, or zones. The inner zona reticularis produces DHEA and DHEA-S and the middle zona fasciculata produces cortisol. The outer zona glomerulosa produces aldosterone which has functions in the renin-angiotensin system and is regulated by other factors than ACTH. The cells in the adrenal cortex are of a single cell type; adrenal cortical cell, but its phenotype can be modulated, by unknown mechanisms, into zona glomerosa cells, zona fasciculata cells and zona reticularis cells [65]. A small amount of the circulating DHEA is produced by the ovary and testis [9,66,67]. It should also be mentioned that DHEA is also synthesized in the brain independent of supply from the adrenal cortex and the gonads [68,69] and the concentration of DHEA in CNS is approximately 6-8 times higher than serum concentrations [70].

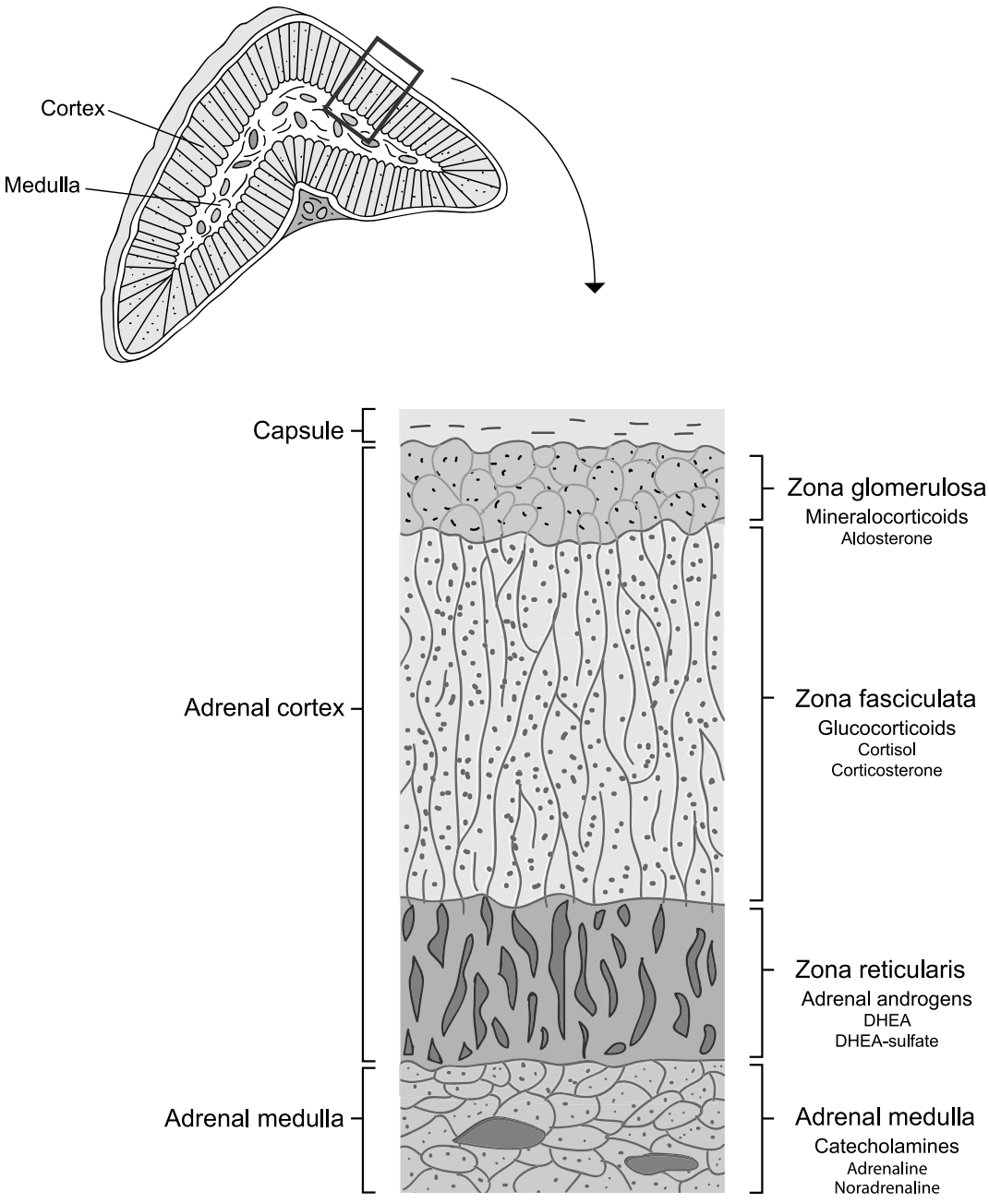

Figure 1. The adrenal gland and its different parts. The adrenal cortex consists of three layers, or zones. The zona reticularis produces adrenal androgens (DHEA and DHEA-S), the zona fasciculata produces glucocorticoids (cortisol) and the zona glomerulosa produces mineralocorticoids (aldosterone). The adrenal medulla secretes adrenaline and noradrenaline.

Regulation of synthesis

As mentioned earlier in the introduction, production of DHEA and DHEA-S is regulated by ACTH [9]. When ACTH reaches the adrenal cortex through the blood stream, it binds to cell-surface receptors of the adrenal cortex. ACTH then stimulates an increase of the steroidogenic acute regulatory protein (StAR), which carries cholesterol from the outer to the inner mitochondrial membranes [71]. Here, the cholesterol is converted to pregnenolone by the cholesterol side chain cleavage enzyme (CYP11A1). Pregnenolone is then further converted in the adrenocortical cells in several steps into different adrenocortical hormones depending on the enzymatic levels within the cells [72,73]. The unique enzymatic phenotype of the zona reticularis cells is a high expression of 17α-hydroxylase/17,20 lyase (CYP17A1), cytochrome b5 (CYB5), DHEA sulfotransferase (SULT2A1), and absence of 3-β-hydroxysteroid dehydrogenase (3β-HSD) [74-76]. This enzyme pattern gives the cells the ability to produce large quantities of DHEA and DHEA-S. CYB5 stimulates the 17,20 lyase activity of CYP17A1 [77] and thereby the formation of DHEA and DHEA-S, by leading the precursors into that pathway. The molecular difference between DHEA and DHEA-S is that DHEA-S has a sulphate molecule attached. SULT2A1 converts DHEA to DHEA-S [75] and steroid sulfatase (STS) can convert DHEA-S back to DHEA [78]. In zona fasciculata cells, there is a high expression of CYP17A1 and abundant 3β-HSD, but absence of CYB5 and SULT2A1, which gives the zona fasciculata cells the ability to produce glucocorticoids [65]. This process of steroid genesis is rapid: within a few minutes from the release of ACTH from the pituitary DHEA, DHEA-S and cortisol are produced. While regulation of cortisol has a negative feedback function, regulation of DHEA and DHEA-S does not. It should be mentioned that additional hormones have been suggested to function as regulators of DHEA and DHEA-S production [79-81], but this is yet to be proven.

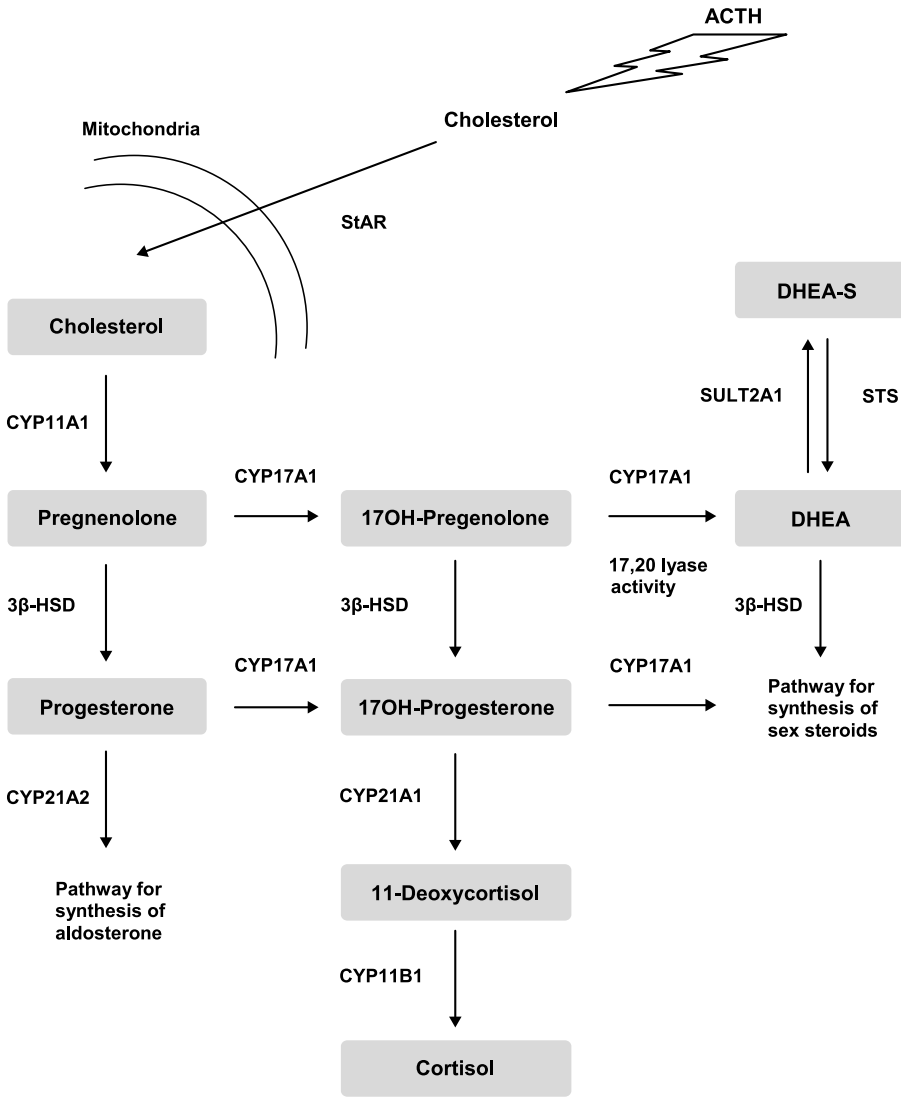

Figure 2. Overview of the different steps in the biosynthesis of DHEA, DHEA-S and cortisol. ACTH stimulates an increase of the steroidogenic acute regulatory protein (StAR), which in turn carries cholesterol from the outer to the inner mitochondrial membranes. Cholesterol is then converted in several steps into different hormones depending on the enzyme levels within the cells. Enzymes are indicated by above and aside arrows.

Circulating DHEA and DHEA-S

When DHEA and DHEA-S are secreted into the blood stream, they are carried bound to albumin. In its sulphated form, DHEA is the most abundant steroid hormone in the circulation. Circulating DHEA-S can be converted to DHEA and vice versa [82]. Concentrations of DHEA-S are between 250 and 500 times higher than the concentrations of DHEA, in women and men respectively [83]. This difference in concentrations between DHEA and DHEA-S depends mainly on the fact that DHEA-S is only slowly cleared from the blood with a clearance rate of 13L/day, while DHEA is rapidly cleared at a rate of approximately 2000L/day [84]. Therefore, DHEA-S has a half-life of 10 to 20 hours while the half-life of DHEA is 1 to 3 hours [82]. The reference interval for DHEA concentration is 2–30 nmol/l (0.60-7.78ng/mL) [85]. Reference intervals for DHEA-S concentrations are 0.76-17 µmol/l (28-640 µg/dL) in men and 0.27-10.3µmol/l (10-380 µg/dL) in women [86]. Thus, men have higher circulating DHEA-S levels than women. The mechanism responsible for this sex difference is not clear.

Diurnal variation of DHEA levels

It is well known that cortisol exhibits a marked diurnal rhythm including a rapid increase in levels just before awakening. The levels peak around 30 minutes after awakening and decline during the day to reach their lowest levels in the evening and during sleep [87]. Diurnal variation of DHEA exhibits a similar pattern as cortisol secretion [88-90]. Thus, DHEA levels are highest in the morning. DHEA-S levels are considered to not have a diurnal variation. However, some studies have reported higher DHEA-S after awakening [91,92]. DHEA-S secretion may be higher in the early the morning but this may not have any major effect on the concentrations, since the circulating DHEA-S levels are very high.

Levels of DHEA and DHEA-S are highly age dependent

During the fetal development, large amounts of DHEA and DHEA-S are produced by the adrenal cortex. The inner zone in the adrenal cortex that produced DHEA and DHEA-S in the fetus goes through a regression process after birth. Consequently, DHEA and DHEA-S levels fall rapidly after birth. The levels remain low until the age of 6-7 years [93,94]. At that time, the zona reticularis layer develops, and the levels start to rise, an occasion called adrenarche. The levels peak at the age of 20 to 30, and thereafter the levels progressively decline with increasing age [86,93,95]. Serum levels of DHEA and DHEA-S in an 80-year-old are only 10-20 % of their peak levels [95,96]. Between the ages of 30 and 50, which is the age range of most of the study

participants in this thesis, DHEA levels decline by 30-50% [85]. In contrast to DHEA and DHEA-S, cortisol levels remain relatively stable during adult life [97]. The dissociation between DHEA/DHEA-S and cortisol in ageing shows that the age-related decline in DHEA and DHEA-S is not caused by changes in ACTH levels. Long-term changes of DHEA-S and DHEA levels are modulated by the number of zona reticularis cells and the enzymes levels (3β-HSD, CYP17A1, CYB5 and SULT2A1) within the cells [79]. Thus, ageing is associated with a reduced number of zona reticularis cells [98] as well as shifted enzymatic activity within the zona reticularis in a way that the capacity to produce DHEA and DHEA-S is reduced (decrease in 17,20-lyase activity) [99]. Biological ageing and increasing risk for age-related diseases and disorders are in part due to this progressive decline in DHEA and DHEA-S levels that occur with increasing age.

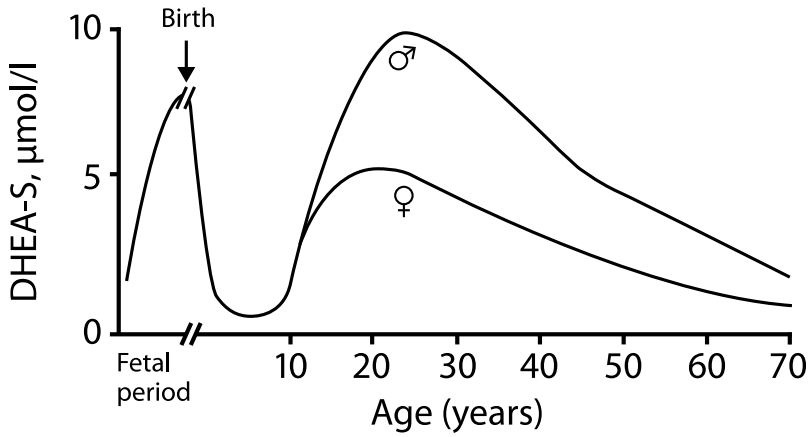

Figure 3. Variation in circulating dehydroepiandrosterone sulphate (DHEA-S) levels throughout life. The DHEA-S levels rise during fetal development with a peak during the last weeks of pregnancy. After birth, the levels decline rapidly. The levels remain low until the age of 6-7 years whereafter the levels start to rise (adrenarche). The levels peak at age 20 to 30, and thereafter the levels progressively decline. The DHEA-S levels during adult life are higher in men than in women. Reproduced from Rainey et al [76].

Functions of DHEA and DHEA-S

DHEA serves as a precursor to the sex steroids testosterone and estradiol. Estradiol and testosterone have, besides their functions in reproduction, anabolic functions, and DHEA and DHEA-S levels therefore indirectly affect these functions. Since no specific DHEA receptor had been found until quite recently, it was believed that DHEA functioned exclusively as a precursor, and thus, its effects were meditated only through conversion to the sex steroids, which in turn mediate their effects by binding to their specific receptors. Recent research has, however, shown that in addition to mediating their effects via transformation into androgens and estrogens, DHEA and DHEA-S are active hormones with effects on their own and that DHEA mediates its action via several nuclear and cell surface receptors [100,101]. DHEA and DHEA-S have pleiotropic beneficial effects. DHEA and DHEA-S have anti-glucocorticoid effects. For example, DHEA protects against the glucocorticoid-induced involution of the thymus [102] and the neurotoxic effects of corticosterone [68] [100]. This is partly due to the antioxidative properties of DHEA and DHEA-S that protect the cells from oxidative stress. For example, DHEA is shown to protect the brain cells against oxidative stress [103] and reduce serum lipid perioxidation [104-106]. DHEA and DHEA-S are not antioxidants but enhances the activity of antioxidant enzymes. DHEA and DHEA-S have been shown to be immune-enhancing [107,108] and to have anti-inflammatory properties [109,110]. For example, DHEA has been reported to increase the number of monocytes and NK cells [107] and to inhibit the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and the tumor necrosis factor (TNF-α) [109,110]. DHEA positively modulates endothelial function, partly by stimulating the production of nitric oxide (NO) in the endothelial cells, which in turn have several beneficial effects on the cardiovascular system. DHEA and DHEA-S play an important role in the regeneration of tissues in the body [52]. For example, DHEA accelerates healing of wounds in the skin [111]. DHEA and DHEA-S seem to also have beneficial effects on mood [112]. Taken together, the functions of DHEA and DHEA-S are protective, regenerative and important for the maintenance and restoration of health. Therefore, DHEA-S and DHEA levels could be used as markers of regenerative and protective (anabolic) activity.

Associations between DHEA and DHEA-S and health

As stated previously in the introduction, ageing is associated with lower levels of DHEA and DHEA-S. Low levels of DHEA and DHEA-S are also associated with adverse health, independently of age. Having the functions of DHEA and DHEA-S in mind it is not surprising that their levels have been

shown to be associated with a wide range of health consequences. Low levels of DHEA and DHEA-S have been shown to be associated with both subjective perception of poor health [113] and with different disease states, for example, depression [114,115], low-back pain and slow rehabilitation of low-back pain in women [83,116,117], frailty in older men and women [118], bone loss in postmenopausal women [119], rheumatoid arthritis [120], cardiovascular disease [121,122], and all-cause mortality in elderly men [123].

Does psychosocial stress affect DHEA and DHEA-S levels?

As mentioned previously, acute psychosocial stress induces elevated cortisol levels, in response to ACTH. Since DHEA and DHEA also are secreted in response to ACTH, it is reasonable to believe that acute psychosocial stress also increases the DHEA and DHEA-S levels. The existing studies measuring DHEA or DHEA-S during acute stress report elevated levels in response to the stressor. However, the knowledge of the DHEA and DHEA-S response to acute psychosocial stress is limited since published studies are few [124-127]. Considering the functions of DHEA and DHEA-S, acute stress-induced increase in DHEA and DHEA-S secretion has been suggested to play a protective role during acute stress, as an antagonist to the effects of cortisol [124,128]. However, factors that affect the response are unknown.

Although DHEA and DHEA-S are regulated by ACTH, dissociations between DHEA/DHEA-S levels and cortisol are observed during ageing and adverse health. Thus, lower DHEA and DHEA-S production is seen in the face of normal or elevated cortisol production. Even among apparently healthy individuals within the same age group, there are inter-individual variations in the DHEA and DHEA-S levels. Genetics account for some of these differences [129] but environmental and lifestyle factors could also be important. Long-term psychosocial stress may be one factor that negatively affects DHEA and DHEA-S levels and explains some of these differences. The relationship between long-term psychological stress and DHEA-S or DHEA levels has been investigated in different ways, but the published studies are few and some observations of these studies contradict each other. Reduced DHEA and DHEA-S levels have been reported in association to prolonged psychosocial stress [130-132], but elevated levels have also been reported [133]. Furthermore, some studies do not show any clear association in any direction [134,135]. If DHEA-S and DHEA levels are reduced in individuals who are exposed to long-term stress, low DHEA-S and DHEA

levels could constitute one link between psychosocial stress, ill health and accelerated ageing.

AIM

The overall aim of this thesis is to investigate the effects of psychosocial stress on serum levels of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S) in otherwise healthy men and women.

The specific aims of Paper I-III are:

Paper I Investigate the effects of acute psychosocial stress on serum levels of DHEA and DHEA-S.

Paper II Investigate the effects of long-term psychosocial stress on the capacity to produce DHEA and DHEA-S during acute psychosocial stress.

Paper III Investigate the effects of long-term psychosocial stress on serum basal levels of DHEA and DHEA-S.

METHOD

This thesis is based on data from two different studies. Paper I and Paper II are based on an experimental study, while Paper III is based on an explorative cross-sectional study.

Participants

In both studies, healthy subjects were recruited either from a cohort study surveying psychosocial work environment and health, or through advertising in a local daily newspaper. In both studies, inclusion was partly based on self-reported level of perceived stress using a single item question from the General Nordic Questionnaire for Psychosocial and Social Factors at Work (QPS Nordic) instrument [136]: "Stress means a situation in which a person feels tense, restless, nervous, or anxious, or is unable to sleep at night because his/her mind is troubled all the time. Do you currently feel this kind of stress?" The response was recorded on a five-point scale varying from "not at all" to "very much." In **Paper I**, thirty-nine healthy subjects (20 men and 19 women, mean age 37.5 years, SD 5 years), participated in the study. To participate in the study, subjects had to be between 30 and 50 years, and only individuals reporting "no stress symptoms at all" or "very little stress symptoms" on the single perceived stress item question [136] were included. These criteria were selected in order to avoid inclusion of individuals suffering from chronic stress symptoms, since this group of participants was originally intended to serve as a healthy control group to be compared with patients with stress-related exhaustion. In **Paper II**, the same participants as in Paper I was included except for three participants as their data on long-term stress (SE questionnaire) was missing. Thus, in Paper II, 36 healthy subjects (19 men and 17 women, mean age 37 years, SD 5 years), were included. In **Paper III**, a selection of participants from a study with 200 otherwise healthy individuals (50 % men) in the age group of 25 to 50 years was included. In that study, the single item question was used in the inclusions procedure to ensure that participants varied in terms of degrees of perceived stress; 40 participants (20 men, 20 women) were selected from each of the five "stress categories" to be included. Among the 200 participants, 183 individuals (91 men and 92 women) had data regarding perceived stress at work (see section on Scoring of perceived stress). Of these 183 individuals, 172 individuals had serum samples stored in −80º C freezer, available for analysis. Of these 172 individuals, the individuals with the lowest and highest perceived stress at work scores (highest and lowest

quartiles) were selected as Non-stressed group (n=40) and Stressed group (n=41) respectively and were included in **Paper III**. Before inclusion of participants in the studies that this thesis is based on, the subjects underwent a screening test, including anthropometric measure-ments and obtaining blood samples to ensure the following exclusion criteria; having a body mass index less than 18.5 kg/m^2 or over 30 kg/m^2, high blood pressure (SBP above 160 mmHg or DBP above 90 mmHg), current infection, folate (vitamin B12) deficiency (established by measuring homocysteine levels), known systemic disease such as diabetes or thyroid disease or known psychiatric disease. Women taking estrogens, nursing, pregnant and postmenopausal women were not included. Subjects who were taking psychoactive medications or any medications that could affect the HPA axis function were excluded.

Study procedures

Within six months after screening, the participants in **Paper I and II** underwent the Trier social stress test (TSST), a standardized laboratory stress test that was set up according to the original design by Kirschbaum and co-workers [137]. The stress task in TSST consists of a simulated job interview and a mental arithmetic task, both in front of a committee (two men and one woman), a video camera, and a microphone. The total test time for each subject was two hours, including preparations and measurements after completing the test. The test procedure was conducted between 1300h and 1700h. At arrival, an intravenous catheter was inserted in the subject's forearm (−30 time point). The first blood sample was drawn at the −10 time point. The next blood sample was drawn immediately before the TSST started (0 time point). Between these two measurements, the participants rested (approximately seven minutes). The stress test started with an introduction of the task and the participants were asked to prepare a speech for a simulated job interview (10 minutes). After this, the participants were exposed to the simulated job interview (five minutes) and thereafter performed a mental arithmetic task (five minutes). Immediately after the end of the stress test (the +20 time point), a third blood sample was drawn. Thereafter, participants rested (recovery period of total 30 minutes), and after 10 and 20 minutes rest, the fourth and fifth blood samples were drawn (+30 and +40 time points). A final blood sample was drawn after the rest (+50 time point). A total of 122 ml blood was collected from the participants during the TSST. Blood samples were collected at six time points (−10, 0, +20, +30, +40, and +50; 7ml at each time point) for measurements of plasma ACTH and serum cortisol. Additional blood samples were collected at four of the six time points (−10, 0, +20, +50; 20 ml at each time point) for the

measurement of DHEA and DHEA-S. The samples were collected in two different tubes; pre-chilled tubes containing EDTA and serum separator tubes. After the tubes had been centrifuged, plasma and serum were stored at −80º C until assayed.

The participants in **Paper III** underwent several tests, including blood sampling and scoring of perceived stress. The tests were performed between 0730h and 1000h. The subjects had fasted overnight and were instructed to abstain from hard physical exercise for 24 hours prior to the tests. At arrival, anthropometry and electrocardiography were performed approximately 30 min prior to the blood sampling. A single blood sample on each participant (50 ml) was drawn. The samples were collected in two different tubes; pre-chilled tubes containing EDTA and serum separator tubes. Plasma and serum were separated by centrifugation, and the samples were stored at −80º C until assayed. After blood sampling, the participants answered a battery of questionnaires, including the Stress-Energy Questionnaire.

The Stress-Energy Questionnaire

Scoring of perceived long-term stress was performed using the Stress-Energy (SE) Questionnaire [138]. This questionnaire has been used in several studies on occupational stress [138-142]; and is a valid tool for measuring stress at work [143]. In this questionnaire, the participants rate how much they agree to 12 different items using a six graded response scale ranging from *not at all* to *very much* (0-5). Thus, they rated how much they agree on how well the items describe how they felt during the previous week. Six of these 12 items measure stress level; *stressed*, *tensed*, *under pressure*, *relaxed*, *calm* and *rested* (the response scale was reversed for the three latter items). The scores from these items were summed up and a mean score was calculated for each participant. In **Paper II**, the participants were divided into three groups (tertiles) based on their mean scores; Low stress (0-1.17), Medium stress (1.50-2.17), and High stress group (2.33-3.67). In the inclusion procedure in **Paper III**, mean scores were used, as described, in the selection of participants, in order to perform a comparison between non-stressed and stressed individuals. The individuals with the highest and lowest scores (highest and lowest quartiles) were included and constituted the non-stressed (0-1.17) and stressed group (3-4.83) respectively. In **Paper II**, the participants answered the questionnaire before performing the stress test (TSST). In **Paper III**, the participants answered this questionnaire after the blood sampling.

Hormone assays

In **Paper I and II**, plasma concentrations of ACTH were measured by immunoradiometric assay (limit of detection, 0.4 pmol/L) (CIS bio International, Gif-sur-Yvette Cedex, France). Serum concentrations of cortisol were measured by electrochemoluminescence immunoassay (limit of detection, 20 nmol/L). In **Paper I, II and III**, serum concentration of DHEA was determined using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method (limit of quantitation, 175 pmol/L) [144,145]. Serum concentration of DHEA-S was measured by radioimmunoassay (RIA) (limit of detection, 0.14µmol/L, Diagnostic Products Corporation, Los Angeles, CA) in **Paper I and II** by quantitative electrochemiluminescent immunoassay (limit of quantitation, 3.46 nmol/L Roche Diagnostics Corporation, Indianapolis, IN) in **Paper III**.

Data handling

In **Paper I and II**, hormonal baseline values were calculated as means of the values determined at the -10 and 0 time points. The peak levels of DHEA, DHEA-S, ACTH and cortisol were identified for each participant (at either +20 or +50 time point for DHEA and DHEA-S, and between the +20 and +50 time points for ACTH and cortisol). Delta values (acute stress-induced increase) of the concentrations of DHEA, DHEA-S, ACTH and cortisol were calculated for each participant, defined as the difference between their peak level and their baseline level. In addition, in **Paper I**, the percentage change in concentration of each of the hormones was calculated (difference between baseline and after stress peak).

Statistical analysis

Logarithmic transformation was used for the variables that showed a non-normal distribution and log values were used in the analyses. For all statistical tests, the level of significance was set at $p < 0.05$. In **Paper I,** mixed "between-within" analysis of variance (ANOVAs) was performed to assess the effect of acute stress on serum concentrations of DHEA and DHEA-S, and whether the DHEA and/or DHEA-S response differed between men and women. DHEA and DHEA-S levels at baseline, the +20, and the +50 time points were used in the analysis (time as within variable) and men and women were compared (sex as between variable). Pearson correlation analysis was performed to assess associations between the magnitude of the stress-induced increase in ACTH and stress-induced increase of DHEA and

DHEA-S (delta values). Pearson correlation analysis was also performed between age and delta values of DHEA and DHEA-S to assess whether age influence the capacity to produce DHEA and DHEA-S during acute stress. In **Paper II,** analyses of covariance (ANCOVAs) were performed to investigate the effect of perceived long-term stress on the response of DHEA and DHEA-S and the ratio between cortisol and DHEA-S response. Hormone response was entered in the models as dependent variable and the variable Stress level group was entered as a predictor. The Low stress group was selected as reference group. Age was entered as a covariate. Results were presented as percentages difference in dependent variables between the reference stress group (Low stress) and the other stress groups. ANCOVAs were also performed in **Paper III** to investigate the effect of perceived long-term stress on basal DHEA and DHEA-S levels. DHEA-S and DHEA levels were dependent variables in the two models respectively. Stress group (non-stressed vs. stressed) was used as predictor, while age and sex were entered as covariates in the models.

Ethical approval

The two studies that this thesis is based on have been approved by the Regional Ethical Review Board in Gothenburg, Sweden (registration number 154-04 and 157-04), and were conducted according to the Helsinki Declaration. All participants gave a written informed consent before participating in the study.

RESULTS

Paper I

Effects of acute stress on DHEA and DHEA-S levels

DHEA and DHEA-S significantly increased in response to the acute psychosocial stressor, in both men and women, along with significantly increased ACTH, cortisol, heart rate, systolic blood pressure, and diastolic blood pressure. The predominant pattern for DHEA and DHEA-S levels was increased levels directly after the stress test, and reduced levels after 30 minutes of recovery. There were no sex differences in the response pattern. While DHEA-S levels were higher at all time points in men, there were no differences between men and women in DHEA levels during the experiment. The magnitude of increase of DHEA and DHEA-S did not differ between men and women, but there was large inter-individual variation in the magnitude of increase of DHEA and DHEA-S concentrations, especially for DHEA. DHEA levels increased on average by 76% in males (5-196%) and by 75% in females (16-177%). The DHEA-S levels increased on average by 19% in males (5-47%) and by 17% in females (2-40%). The magnitude of the stress-induced DHEA, but not DHEA-S, was significantly associated with the magnitude of the stress-induced increase of ACTH ($r = 0.54$, $p < 0.001$; $r = 0.21$, $p = 0.191$, respectively), thus explaining some of the inter-individual variation. The magnitude of stress-induced increase of both DHEA and DHEA-S levels correlated negatively with age ($r = -0.37$, $p = 0.022$; $r = -0.39$, $p = 0.015$, respectively), thus there was an attenuated response in the older compared to the younger individuals.

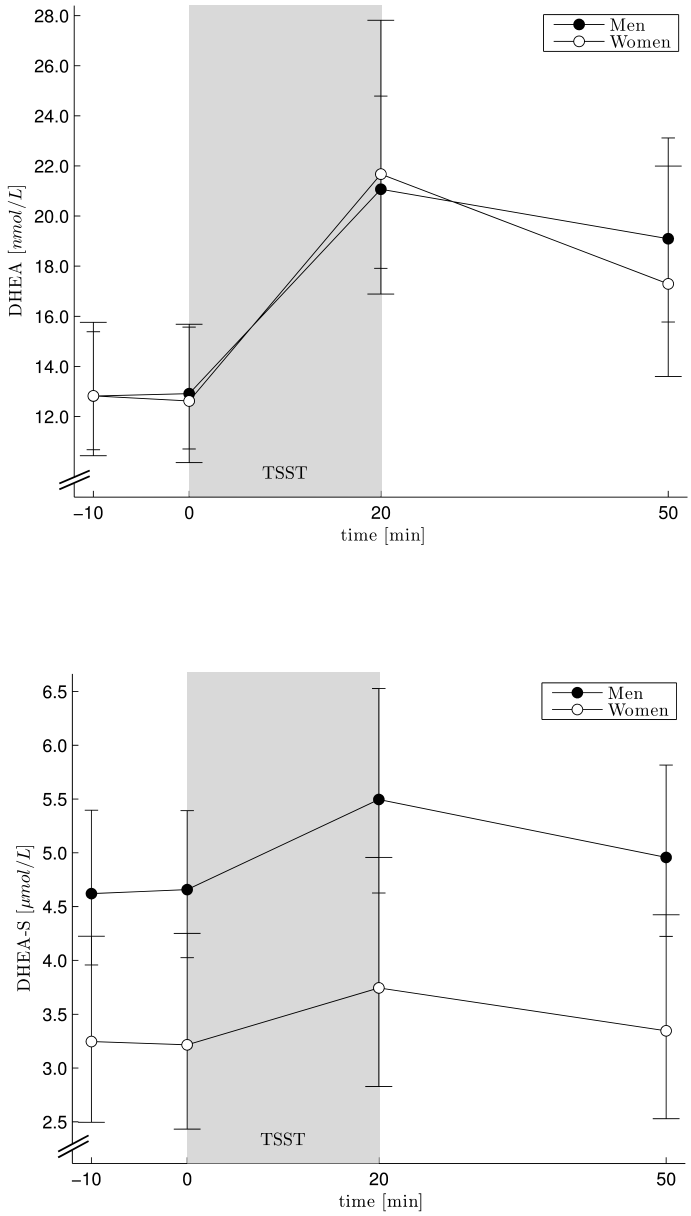

Figure 4. Geometric mean (95% CI) serum concentrations of DHEA and DHEA-S before the stress phase (the −10 and 0 time points), immediately after the stress phase (the +20 time point) and after 30 min of recovery (the +50 time point) in 20 men and 19 women.

Paper II

Effects of long-term stress on the capacity to produce DHEA and DHEA-S during acute stress

The DHEA-S production during acute psychosocial stress was markedly attenuated in individuals reporting prolonged stress. The magnitude of stress-induced DHEA-S increase was on average 50% lower (95 % CI 4-74 %, p = 0.037) in the group of individuals that reported high levels of stress at work and 52 % lower (95 % CI 8-75 %, p = 0.027) in the group of individuals that reported medium levels of stress at work compared to the group of individuals that reported no stress at work, after adjusting for age. There was no difference between the groups in DHEA response. The ratio between the stress-induced increase of cortisol and the stress-induced increase of DHEA-S was on average 137% higher (95 % CI 20–367 %, p = 0.014) in the High stress group compared to the Low stress group, after adjusting for age.

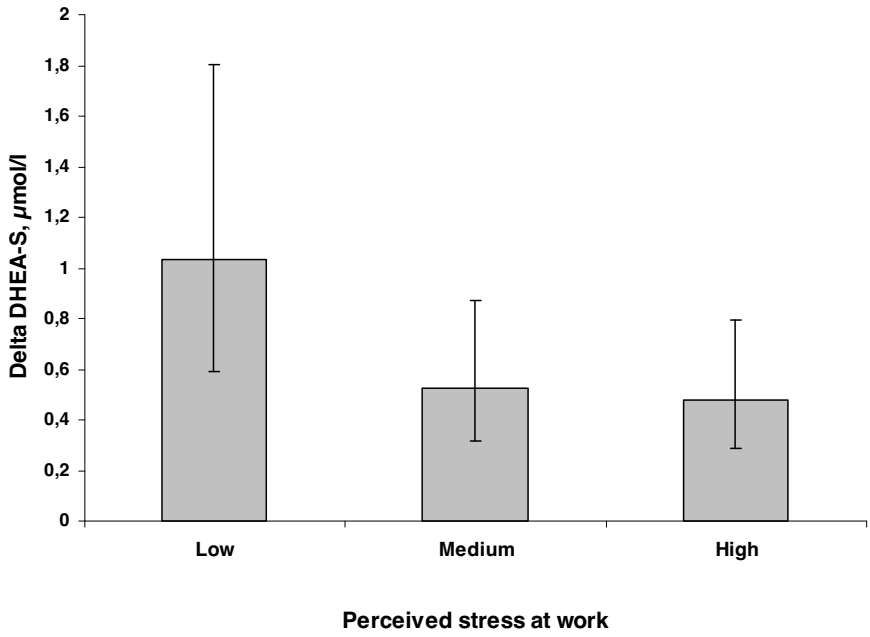

Figure 5. Geometric mean (95% CI) increase in DHEA-S levels during acute psychosocial stress in the three groups of participants with different levels of perceived stress at work (Low, Medium and High).

Paper III

Effects of long-term stress on DHEA and DHEA-S levels

Individuals who reported that they experienced prolonged stress, measured as perceived stress at work, had markedly lower levels of DHEA-S than the individuals who reported no stress. DHEA-S levels were on average 24 % lower (95 % CI 2-50 %, p = 0.029) in the stressed group compared to the non-stressed group, after adjusting for age and sex. There was no difference in DHEA levels between the stressed group and the non-stressed group, after adjusting for age and sex (p=0.348).

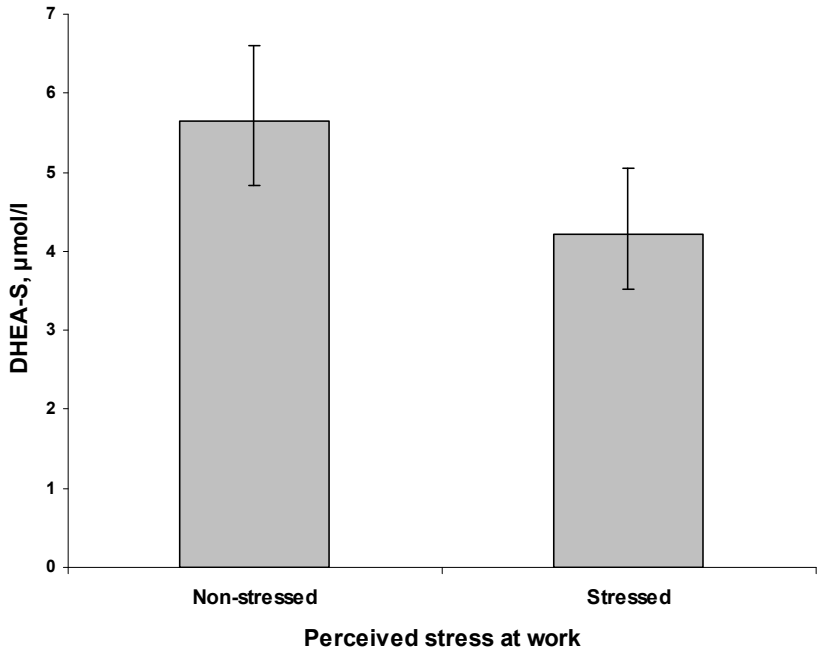

Figure 6. Geometric mean (95% CI) DHEA-S levels in the non-stressed and stressed individuals.

DISCUSSION

The aim of this thesis was to investigate the effects of acute and long-term psychosocial stress on serum levels of DHEA and DHEA-S. The first paper showed that DHEA and DHEA-S levels markedly increase during acute stress. It also showed that the magnitude of increase was dependent on the magnitude of ACTH response and the capacity to produce DHEA and DHEA-S during acute stress reduced with age. The second paper showed that prolonged stress negatively affects the capacity to produce DHEA-S during acute stress. The third paper showed that prolonged stress is associated with markedly lower basal levels of DHEA-S. Thus, the thesis shows that while acute stress elevates DHEA and DHEA-S levels temporarily, long-term stress may reduce the capacity to produce DHEA-S both at rest and during acute stress. The results of this thesis are discussed below in relation to previous findings, the biological significance and mechanisms, and future research directions.

Paper I

DHEA and DHEA-S levels increase during acute psychosocial stress

Paper I showed that DHEA and DHEA-S levels significantly increased in response to the acute stressor, along with significantly increased ACTH, cortisol, heart rate, systolic blood pressure, and diastolic blood pressure. The predominant pattern for DHEA and DHEA-S levels during the TSST was increased levels immediately after the stress, and reduced levels after 30 minutes of recovery. The response of DHEA and/or DHEA-S to acute stress has been investigated in a few studies previously [124-126,146], and similarly, increased levels of these hormones were reported. One of these studies was conducted on women [146]. Paper I in this thesis is the first published paper that included both men and women when evaluating DHEA and DHEA-S levels during acute psychosocial stress. The results suggest that there are no sex differences in the pattern or magnitude of the change of DHEA or DHEA-S levels in response to acute psychosocial stress. In agreement with the fact that men have higher levels of DHEA-S than women, the male participants exhibited higher DHEA-S levels during the experiment.

The magnitude of DHEA increase is associated with ACTH response

There was large inter-individual variation in the magnitude of increase of DHEA and DHEA-S concentrations, especially for DHEA (5 - 196 %). Since short-term changes of DHEA are regulated by ACTH, analysis was performed to investigate whether differences in ACTH response explained some of the inter-individual variation. The magnitude of stress-induced increase of DHEA positively correlated with the magnitude of stress-induced increase of ACTH. Thus, some of the inter-individual variation in DHEA could be explained by differences in the ACTH response, which in turn likely depends on the differences in stress levels perceived during the TSST. While it has been previously shown that administration of ACTH increases DHEA levels in a dose-response manner [147], this is the first time that DHEA response to acute psychosocial stress has been related to the ACTH response.

The capacity to produce DHEA and DHEA-S during acute stress decreases with age

As described in the introduction, DHEA and DHEA-S levels decline progressively with age. It could therefore be hypothesized that the stress-induced DHEA production also reduces in old age. It has been shown that administration of ACTH or CRH increases cortisol levels in old and young subjects likewise whereas the increase in DHEA and DHEA-S is much smaller in the old subjects [99,147-149]. To my knowledge, the relationship between age and psychosocial stress-induced levels of DHEA or DHEA-S has not been previously studied. The results of Paper I showed that the magnitude of acute stress-induced increase in DHEA and DHEA-S is negatively associated with age, thus, the capacity to produce DHEA and DHEA-S during acute stress reduces with age.

Paper II

Long-term stress is associated with attenuated capacity to produce DHEA-S during acute stress

In Paper II, it was shown for the first time that long-term stress was related to markedly attenuated DHEA-S response during acute psychosocial stress. Thus, prolonged stress seems to negatively affect the capacity to produce DHEA-S during acute stress. The significant differences in DHEA-S response that were seen even between the Low stress and Medium stress groups indicates that even relatively low levels of perceived prolonged stress seem to affect the capacity to produce DHEA-S. There was no difference

between the groups in DHEA response. Since the DHEA levels are about 250-500 times lower than the DHEA-S levels, and since DHEA-S can rapidly convert to DHEA and vice versa [82] the non finding in DHEA levels may be of minor importance. Even though the relative change (percentage increase) of DHEA during acute stress is greater than the relative change of DHEA-S, the actual increase of DHEA-S concentration during acute stress is much larger than for DHEA, and the delta DHEA-S accounts for the majority of the total increase in DHEA and DHEA-S together (by average 98 % in this study).

Paper III

Individuals reporting long-term stress had lower levels of DHEA-S

DHEA-S and DHEA levels in individuals who reported perceived stress at work were compared with levels of these hormones in individuals who reported no perceived stress at work. The stressed individuals had markedly lower levels of DHEA-S than the non-stressed individuals. Thus, the stressed individuals had by average 24 % lower levels of DHEA-S than the non-stressed individuals, after adjusting for age and sex. As mentioned several times in the thesis, DHEA-S levels decline with age [86,150]. The individuals who experienced prolonged stress exhibited DHEA-S levels that are more typical of levels observed in older individuals. A 24 % difference in basal DHEA-S levels corresponds to the difference in DHEA-S levels when for instance a 30 year old is compared to a 50 year old. There was a large variation in the degree to which the DHEA-S levels were lower in the stressed individuals (2–50 % lower) as compared to the non-stressed. However, the result indicates that stressed individuals exhibit DHEA-S levels that are normal at older ages than their own age.

In studies on effects of prolonged or chronic stress, preferably DHEA-S levels are measured rather than DHEA levels, since they are more stable and show no or small diurnal variation as DHEA does. However, besides DHEA-S, DHEA levels were also measured in the study on the effects of prolonged stress. DHEA levels did not differ between stressed and non-stressed individuals. One plausible reason for that could be that we collected blood samples in the morning and thus the morning secretion of DHEA [88] could have affected the results. Since DHEA levels are about 250-500 times lower than DHEA-S levels, and since DHEA-S can rapidly convert to DHEA and vice versa [82], the non finding in DHEA levels may be of minor importance.

As mentioned in the introduction, the relationship between prolonged psychological stress and DHEA-S or DHEA levels has previously been investigated in different ways but the number of studies is relatively small and some observations of these studies contradict each other. Reduced levels of DHEA and DHEA-S have been reported in association to prolonged psychosocial stress [130-132], but elevated levels have also been reported [133]. Furthermore, some studies do not show any clear association in any direction [134,135]. One strength of Paper III compared to some of the previous studies is that individuals who report a high level of stress are contrasted with individuals on the other end of the distribution: those reporting no stress.

Biological significance

It is well known that long-term stress can cause and contribute to a wide range of psychological and somatic conditions and accelerate aging. It is also known that DHEA and DHEA-S have protective and regenerative (anabolic) effects and functions [68,100,151], and that low DHEA-S and DHEA levels are associated with higher risk for adverse health. Lower levels of DHEA-S, as seen in the (chronically) stressed individuals, thus lead to increased risk for adverse health and accelerated ageing, at least if high levels of perceived stress remains high for longer periods.

Acute stress-induced DHEA and DHEA-S have been suggested to play a protective role during acute stress, as an antagonist to the negative consequences of stress, in particular the effects of cortisol [124,128]. This includes beneficial effects on mood and behaviour during acute stress [124,125]. The markedly smaller production of DHEA-S in individuals who experience long-term stress thus indicates that chronically stressed individuals have lesser protection during acute stress situations. The negative association between age and acute stress-induced DHEA and DHEA-S increase also indicates that older individuals have lesser protection during acute stress than younger.

The findings that long-term stress is associated with lower capacity to produce DHEA-S (during acute stress and during basal conditions) indicate that lower levels of DHEA-S may constitute a link between psychosocial stress, ill health, and accelerated ageing. The study population consists of relatively healthy individuals. It can therefore be said that the reduced DHEA-S production capacity seen in the (chronically) stressed individuals is an early physiological effect of long-term stress.

Mechanisms

As described in the introduction, DHEA and DHEA-S are produced in the zona reticularis in the adrenal cortex in response to ACTH. Thus, short-term changes in DHEA and DHEA-S levels, such as those occurring during acute stress, depend on changes in ACTH levels. CRH is released from the hypothalamus when we perceive a situation as stressful. CRH in turn stimulates the release of ACTH from the pituitary. When ACTH reaches the adrenal cortex through the blood stream, the adrenal cortex produces DHEA, DHEA-S and cortisol. The results of this thesis suggest that the capacity to produce DHEA-S is set at lower level if the individual experiences prolonged stress, both during rest and during acute stress. Long-term changes in DHEA and DHEA-S levels are modulated by the number of zona reticularis cells and enzyme levels (3β-HSD, CYP17A1, CYB5, and SULT2A1) within the cells [75]. Thus, by unknown mechanisms, long-term stress likely affects the enzyme levels in a way that the capacity to produce DHEA and DHEA-S decreases and possibly the number of zona reticularis cells also decreases. The attenuated DHEA-S production shown in individuals reporting perceived stress at work may indicate that during prolonged stress, steroid biosynthesis may be shifted from biosynthesis of adrenal androgens to corticosteroid pathways ensuring maintained production of cortisol, which is essential during exposure to stressors. Insulin has been reported to inhibit the production and increase clearance of DHEA and DHEA-S [152]. Since long-term stress can lead to insulin resistance and higher insulin levels [153], insulin may play a role behind the low DHEA-S levels in stressed individuals. Lower DHEA-S levels in stressed individuals might also mirror an increased utilization of DHEA and DHEA-S. It should be noted that since a small amount of DHEA is produced by the ovary and testis [9,66], DHEA reductions could possibly also mirror the production capacity in the gonads.

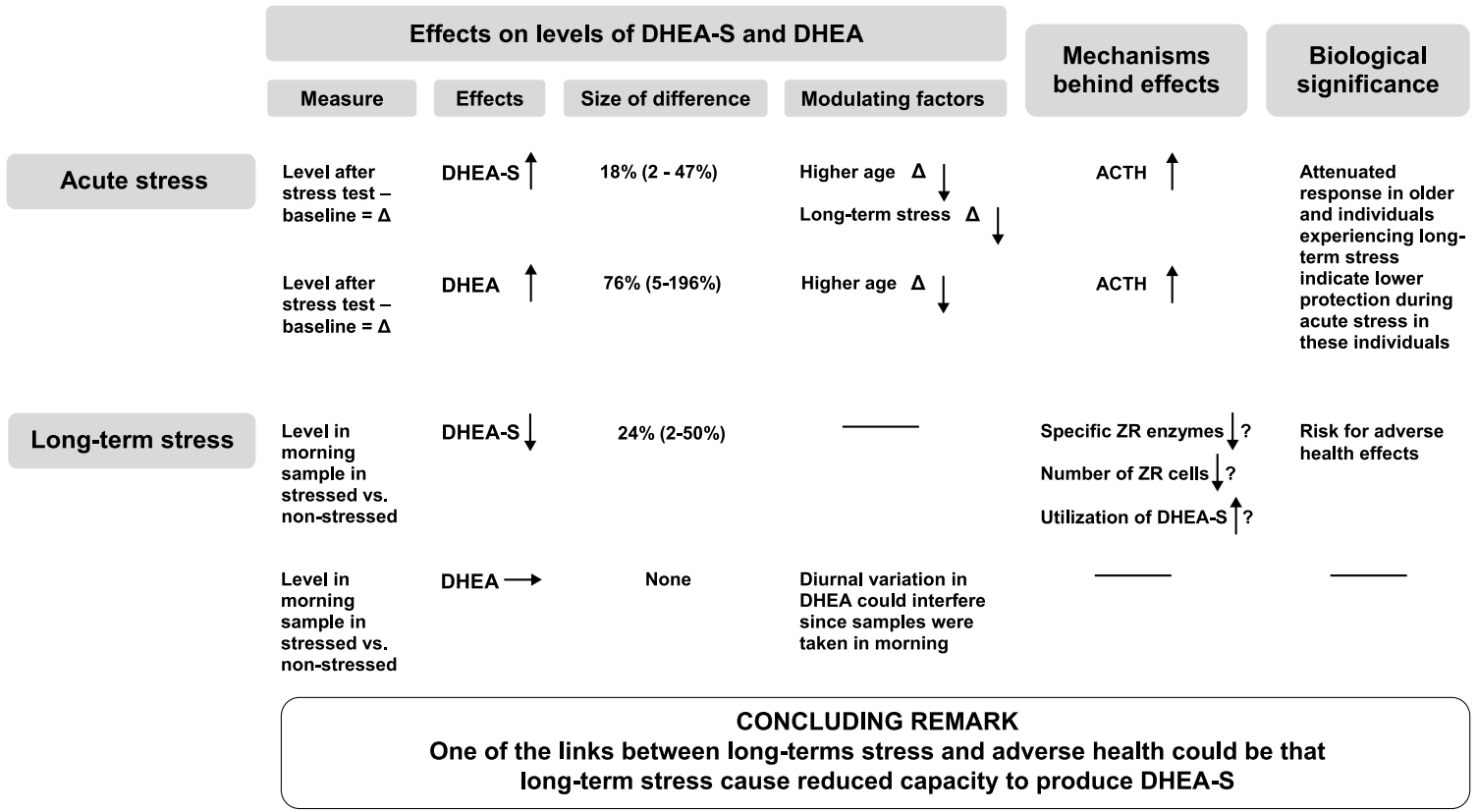

Figure 7. Summary of the thesis

Can decreased capacity to produce DHEA-S be reversed?

One important question is whether, how, and to what extent the DHEA-S and DHEA production could be reversed, normalized and improved. Some studies have used DHEA-S levels as a marker of positive outcome (increased anabolic activity) of interventions aiming to reduce psychological stress or improve psychosocial conditions [154-160], and some interventions have been shown to be protective. There are studies that show that DHEA-S levels could markedly be improved as an effect of stress-reducing interventions [156,158]. Furthermore, there are studies that indicate that long-term practice of methods that reduce stress (e.g. meditation and physical activity) may increase DHEA-S levels or prevent the age-related decline in DHEA-S production [161-163]. Thus, the individuals who experienced prolonged stress (and exhibited attenuated capacity to produce DHEA-S) in the present thesis, could potentially improve their DHEA-S levels if the perceived stress decreases and suitable resources for recovery are provided.

Methodological considerations

There are some topics that need to be considered when interpreting the results of this thesis.

Study population

The thesis is based on data from two different studies. Paper I and Paper II are based on an experimental study of acute stress responses (n = 39), while Paper III is based on a cross-sectional explorative study originally intended to explore the relationship between self-rated perceived stress and plausible biological markers of stress (n = 200). In both studies, subjects were recruited either from a cohort study surveying psychosocial work environment and health, or through advertising in a local daily newspaper. All subjects were living in western Sweden within or around the Gothenburg area.

As also mentioned in the method section, only individuals reporting “no stress” or “very little stress” on a single screening question were included in the experimental study that Paper I and Paper II are based on. This criterion was used in order to avoid inclusion of individuals suffering from chronic stress symptoms, since this group of participants was originally intended to serve as a healthy control group to be compared with patients with stress-related exhaustion. This might be a problem when, as in Paper II, the goal is to study the effects of long-term stress on acute stress responses. However,

the scores from the questionnaire measuring long-term stress showed that the participants varied from perceiving no stress to perceiving relatively high stress levels (see Measurement of long-term stress below) and the effects of long-term stress on acute responses could therefore be studied. There was a period of approximately least six months between screening/inclusion with the single perceived stress item question until the participants performed the stress tests and concomitantly reported their long-term stress levels. Accordingly, this might explain why perceived stress level could have changed during this period.

Inclusion to Paper III was done to ensure that the effects of long-term stress could be investigated. First, in the original study aimed to investigate biological markers for stress, a single item question measuring perceived stress levels was used in the inclusion procedure to ensure that participants varied in terms of degrees of perceived stress; 40 participants (20 men, 20 women) were selected from each of the five stress categories to be included in the initial sample of 200 individuals. Of these, 172 individuals had available data on perceived stress at work and an extra blood sample in the freezer (in which DHEA and DHEA-S could be measured). Those with the lowest and highest perceived stress at work scores (highest and lowest quartiles; n = 41, n = 40; respectively) were included in Paper III. Thus, individuals reporting a high level of work-related stress are contrasted with individuals on the other end of the distribution, those reporting no work stress at all.

The cohort study that the participants were recruited from consisted of a population of health care workers and employees at The Swedish Social Insurance Agency. The subjects that were recruited through the advertisement in the local paper had different kinds of jobs and a few of them were students or unemployed. Only working individuals were included in Paper II and III since they investigate the effect of perceived stress at work. In the study investigating the effects of long-term stress, some background information was collected. The majority (60-70%) of the participants (in both the stressed and non-stressed groups) had jobs that require higher education, were physically active at least to some degree and very few of them were nicotine users. The fact that the majority of the participants had jobs requiring higher education indicates that the sample consisted of individuals with higher rather than lower socioeconomic status. Individuals having lower socioeconomic status may be more vulnerable to stressors since they are more likely to have less beneficial coping patterns, smaller social networks, less social support, fewer economic resources and in general, a lifestyle that does not buffer the effects of stress (for example a sedentary lifestyle)

[164,165]. This suggests that if the studies had included more individuals with lower socioeconomic status, the effects of long-term stress might have been even larger.

One of the aims of the screening procedure was to exclude individuals with diseases, thus only relatively healthy individuals were included. Therefore, it can be said that the reduced DHEA-S production capacity seen in the (chronically) stressed individuals is an early sign of the effects of prolonged stress. Although it is plausible to believe that the capacity to produce DHEA-S is reduced also in individuals who suffer from stress-related disorders, the results cannot be generalized to groups other than those of healthy individuals.

Another issue is the age and BMI range of the study population. The acute stress study (Paper I and II) was conducted on individuals between 30 and 50 years old. The study that explored the effects of long-term stress (Paper III) was conducted on individuals between 25 and 50 years. These limited age ranges were determined for inclusion in the original projects because the original projects aimed to study physiological stress responses and effects without having to account for age differences. Despite the limited age groups, age differences could be observed and reported in this thesis, which is an advantage. However, future studies on DHEA and DHEA-S responses to acute and long-term stress would benefit from also including adults older than 50 years. Furthermore, as no obese or underweight individuals were included in the studies (depending on the inclusion criteria), we cannot generalize the results to these groups of individuals.

Measurement of acute stress

Acute stress responses were studied in this thesis using Trier Social Stress test. TSST is a well-known standardized laboratory stress test [137] which is conducted to study acute physiological (and psychological) stress responses. TSST is validated; it clearly induces temporary stress in participants. Unfortunately, perceived stress and coping patterns during the stress test were not assessed. This information could possibly have extended the conclusions.

Measurement of long-term stress

The Stress-Energy Questionnaire was used to measure prolonged perceived stress. This questionnaire has been used in several studies on occupational stress [138-142] and is a valid tool for measuring stress at work [143]. The Stress-Energy Questionnaire assesses perceived stress and perceived energy at work and leisure during the previous week. In this thesis, it was the

perceived stress scale that was of interest and not the subscale measuring energy level. The mean score of the perceived stress at work was used and not leisure time simply because few individuals reported high levels of stress at leisure time. In the study on the effects of long-term stress on acute DHEA and DHEA-S responses, only two individuals reported stress during leisure time. These two individuals belonged to the High stress group, thus they were not misclassified as non-stressed. In the study on the effects of long-term stress on basal levels of DHEA and DHEA-S, none of the men or women that were classified as non-stressed could be classified as having stress at leisure time. Thus, none of the participants were misclassified as non-stressed. It should be discussed that we cannot define our measure of perceived stress at work during one week as a measure of chronic stress, but it can be considered as prolonged stress compared to an acute stress situation such as TSST, which lasts for 20 minutes. The circumstances during the previous week are likely to reflect normal conditions. It is thus likely that the perceived stress levels during the previous week reflect representative levels of stress. However, we cannot assume that the observations reflect longer exposure to stress, or that the observed inhibited DHEA-S production is an effect of temporary exposure to stress at work. Future studies should measure long-term stress with other tools as well that capture longer periods and follow up stress levels and DHEA-S levels prospectively.

DHEA and DHEA-S measurement

In the studies on acute stress responses (Paper I and II), DHEA and DHEA-S levels were measured at four time points. The two first samples were taken before the stress test started and a mean of these two was calculated and used as the baseline level. The third sample was collected immediately after the stress test and the fourth sample was collected after 30 minutes of recovery. However, ACTH and cortisol were measured at two additional time points (after 10 and 20 minutes of recovery). The reason for this is that DHEA and DHEA-S measurements were not included in the original study plan, as cortisol and ACTH measurements were. DHEA and DHEA-S measurements were performed on extra samples that had been saved in the freezer. The extra samples were taken only at four time points since larger volume of blood could not be collected from the participants for ethical reasons. If blood samples had been collected at additional time points such as 10 and 20 minutes after the stress test, the likelihood to capture the peak would have increased. The stress test was conducted in the afternoon on all subjects, thus the influence of the diurnal rhythm in hormonal secretion was avoided.

Measuring basal DHEA in morning, as it was done in Paper III, is not optimal, as DHEA has a pronounced diurnal rhythm and exhibits morning

elevation similar to cortisol, which could affect the results. The rationale behind the fact that DHEA was measured only in the morning is that measurement of DHEA and DHEA-S was not part of the original plan of the study (with the initial 200 participants described in the participants section). Thus, only samples taken in the morning were available for this study. DHEA-S levels, on the other hand, are more stable and show less or no diurnal variation and hence the time point of the measurement should not affect the results.

In the studies in this thesis, DHEA levels are measured using Liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS offers analytical specificity and sensitivity superior to that of immunoassays [145]. It has been shown that immunoassays are inaccurate for measurement of steroids in low concentrations (testosterone in women, estradiol and estrone in postmenopausal women and men, DHEA and androstenedione in elderly) [144,145]. In the lab that assayed the samples, LC-MS/MS method for DHEA-S was not available and DHEA-S was therefore measured using immunoassays. However, since the concentration of DHEA-S is very high (much higher than that for the other steroids mentioned above), immunoassay is accurate for the measurement of DHEA-S.

CONCLUSIONS

While acute psychosocial stress increases the levels of DHEA and DHEA-S temporarily, long-term psychosocial stress is associated with lower basal DHEA-S levels and reduced capacity to produce DHEA-S during acute stress. Considering the beneficial functions and effects of DHEA and DHEA-S and the fact that low DHEA and DHEA-S levels are associated with adverse health, **the findings of this thesis suggest that one of the links between long-terms stress and adverse health could be that long-term stress causes reduced capacity to produce DHEA-S.** The study population consists of healthy individuals. It can therefore be said that the **reduced production capacity seen in the (chronically) stressed individuals is an early physiological effect of long-term stress**. The findings enhance the understanding of how long-term psychosocial stress could cause and contribute to disorders and health conditions, and accelerate aging. The findings add one more reason to emphasize the importance of helping a stressed individual to eliminate the stressors and of reducing the perceived stress level, also at an early stage. They also underline that suitable resources should be provided for recovery. The findings support the idea that DHEA-S could more often be used as a biological marker of stress, to reflect anabolic (health promoting) activity, in addition to cortisol and other markers.

FUTURE RESEARCH DIRECTIONS

At present and in the past, when stress physiological responses (acute responses and long-term effects) have been measured, the emphasis has been on various indices of catabolic activity, in particular cortisol levels. As described in the introduction, the increase in catabolic activity that occurs during a stress reaction resulting in mobilization of the body´s energy resources helps us to overcome the stressor but increased catabolic activity also induces bodily processes that, if prolonged, result in negative health consequence. In other words, these processes may cause, contribute to and negatively influence the course of psychological and somatic health conditions. While cortisol levels reflect mainly catabolic activity, DHEA-S and DHEA levels reflect regenerative (anabolic) activity. Thus, DHEA and DHEA-S have opposite roles (protective and regenerative) compared to what cortisol has. The regenerative activity is fundamental for staying healthy and in restoration of health. Unfortunately, when investigating the physiological effects of stress or the physiological effects of stress-reducing interventions, anabolic activity has been examined only in exceptional cases. **I suggest that, when investigating the physiological effects of stress, DHEA-S and DHEA should be assayed in addition to cortisol.** By doing this, the effects of stress on the "healthy" anabolic part are also seen. The effects of stress on DHEA and DHEA-S are, in this thesis, studied in an experimental study (acute responses) and a cross-sectional study (long-term effects). Future studies on effects of long-term stress could also include assessments of DHEA-S prospectively. This would provide knowledge on how DHEA-S levels may fluctuate depending on the stress level.

It is of great importance to know whether the individuals exhibiting reduced DHEA-S production due to long-term psychosocial stress can normalize their levels when stress exposure/experience reduces. Future studies on the relationship between DHEA, DHEA-S and stress should investigate how and to what extent the attenuated DHEA-S production could be reversed, improved, and normalized. **I suggest that future studies on effects and mechanisms of interventions and methods which aim to reduce stress, increase well-being and perception of health include measurement of DHEA and DHEA-S** in evaluation of health consequences. By doing this, researchers can measure whether the body's regenerative activity is restored and improved, which is important in restoring health. Examples of methods and interventions that could possibly help to improve the DHEA and DHEA-S levels are for example physical exercise, meditation, work environment interventions and cognitive and behavioral therapy. DHEA and DHEA-S

levels could also for example be assayed when methods and interventions aiming at improving sleep are evaluated, since recovery during sleep is very important for regeneration and restoration.

As mentioned in the introduction, the decline in DHEA and DHEA-S that occurs during ageing is associated with increased risk of morbidity and even mortality. Therefore, **assaying DHEA and DHEA-S is especially important in studies on elderly populations**. Preventing the age-related decline or improving the DHEA and DHEA-S levels (with above mentioned potential methods for example) is thus especially valuable in the elderly.

In research, there has hitherto been more focus on disease and damaging bodily processes than on health, health promoting and restorative bodily processes. This is the case not only in the stress research field but also in medical and bio-psychological research in general. This likely reflects the view and focus of the majority of researchers and clinical practitioners and probably also stakeholders. **I suggest that health promoting and restorative bodily processes should receive more attention and be given higher priority than they currently have.** Such a focus would benefit public health.

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